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ORIGINAL ARTICLE

Validity and Role of Immunohistochemistry Studies in Prediction and Staging of Malignant Lymphoma Among Iraqi Patients

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ABSTRACT

***Background**: Malignant lymphomas represents a heterogeneous collection of neoplasms characterized by tumor cells that have similarity to mature lymphocytes, histiocytes, or their progenitors. The selection of suitable markers by pathologists is of utmost importance, to achieve an optimal diagnosis , staging and monitoring of patients with lymphoma.*

***Objective**: To assess the validity and role of Immunohistochemistry studies in Prediction and Staging of Malignant Lymphoma among Iraqi Patients.*

***Methods**: A prospective clinicopathological and immunohistochemical study conducted during a period of 20 months, in the years 2021-2023. The study included 42 Iraqi patients with different types of malignant lymphomas. A total of 65 tissue specimen blocks were sectioned and examined utilizing immunohistochemical studies*

***Results**: Hodgkin's (HL) and Non- Hodgkin's (NHL) lymphomas confirmed in 57.1% and 42.9% of cases, respectively. Patients tend to be of older age with a mean age of 47.3 years in HL and 50.6 years in NHL cases. Males were relatively dominant in both groups. Immunoreactivity score (IRS) and Ki-67 proliferative index were good predictor of advanced stage malignant lymphomas.*

***Conclusions**: The immunohistochemical (IHC) studies has a crucial role in the diagnostic process of types and subtypes of lymphomas. Immunoreactivity score (IRS) and Ki-67 proliferative index were good predictor of advanced stage malignant lymphomas for both HL and NHL*

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***Keywords:** Lymphoma, Types, Malignant, Diagnosis, Staging, Immunohistochemistry*

1. INTRODUCTION

Lymphomas develop from abnormal lymphocytes. A distinction is made between around 60 different subtypes of lymphoma - and the possible symptoms are also diverse. Typical symptoms do not always occur, but there are some warning signs. Malignant lymphoma include two main types; Hodgkin's and non-Hodgkin's based on the histological appearance. The NHL are made up of around 40 subtypes, with 80% originating in either mature or immature B cells and the remaining 20% in T and natural killer cells. (1–3). Although malignant lymphoma is considered a rare disease, its incidence has increased in the last 4 decades at a rate of 3-4%. Annually, more than 200000 cases are newly detected with malignant lymphomas Sixty percent of these cases are attributable to NHL, whereas HL accounts for the remaining forty percent. On the other hand, about 3% of all cancer fatalities are attributable to lymphomas worldwide (4). Hodgkin's lymphomas is a possibly curable disease, and the 10-year survival reached about 80%, however, but it leads to recurrence or death in about 20-30% of patients, and even for patients with NHL in advanced stages, a complete cure is also possible (5). Until the end of the 20th century, the therapy of malignant lymphoma consisted mainly of a combination of cytotoxic chemotherapy with or without additional radiotherapy. However, the treatment results were not satisfactory in many cases. Clinically, it exhibits an aggressive progression . Furthermore, other category of NHL lymphoma is the mature B-cell lymphoma (BCL), which also called mantle cell lymphoma, that is cytologically classified as an indolent NHL with an average survival period of three to four years. The low-grade B-cell lymphoma has the most worse prognosis, as majority of patients are diagnosed at an advanced stage The prognosis of mantle cell lymphoma is also poor due to the absence of a known curative treatment (6).Based on morphological criteria, a differentiation is established between HL (presence of Hodgkin's and R-S cells) and NHL where these cells are typically not observed. Despite the low incidence rate of these malignancies they hold substantial significance due to the notable surge in the prevalence of these malignancies throughout the last three decades. Nevertheless, it is important to note that there is a gradual increase in the incidence of newly confirmed HLs. (3,7)

Classification:

Hodgkin's lymphoma: Depending on the histomorphological picture, phenotypes, and molecular features, according to the REAL/WHO, HL can be classified into classic (cHL) which is further classified into nodular sclerosing, Lymphocyte-dominant, Mixed-cellular and Lymphocyte-depleted HL (8,9).

Non-Hodgkin's Lymphoma:

Due to the complexity and diversity, there was no uniform classification for the NHL for a long time. New findings in relation to morphology, cytology, clinical, molecular genetics and immune phenotypes were taken into account (11). According to the WHO classification was published in 2001 (10) and revised in 2016 (11). NHL is classified based on the type of cell from which the malignancy is originated. Almost 10-15% of NHL are originated from T or natural killer (NK) cells while majority of NHL (almost 85-90%) of B-cell origin and they are different in their histomorphological, phenotypes and clinical features (8).

Pathogenesis:

Hodgkin's lymphomas are characterized histologically by the Hodgkin's and Reed-Sternberg cells. These are large blasts and multinucleated giant cells of monoclonal origin (Figure 1). They represent the actual neoplastic cells (12). However, they only make up about 1% of the entire cell picture. The rest is formed by an inflammatory infiltrate, including lymphocytes, monocytes, granulocytes, mast cells and histiocytes (13). The 4 subtypes of classic HL ; nodular sclerosing, rich in lymphocytes, mixed cells and poor in lymphocytes, can be distinguished based on the number of Hodgkin's and Reed-Sternberg cells, cell infiltrate, growth pattern and age of the patients. From other point of view, the classic HL are characterized immunohistochemically by the surface features CD30 and partially CD15. These are absent in the NLPHL. Specific B cell antigens (e.g. CD20, CD79a) are characteristic of NLPHL. The infiltrate can be recognized by small nodular lymphocytes and only occasionally has giant cells with small nucleoli, which are known as "lymphocyte predominant cells" (LP cells) or popcorn cells (14,15)

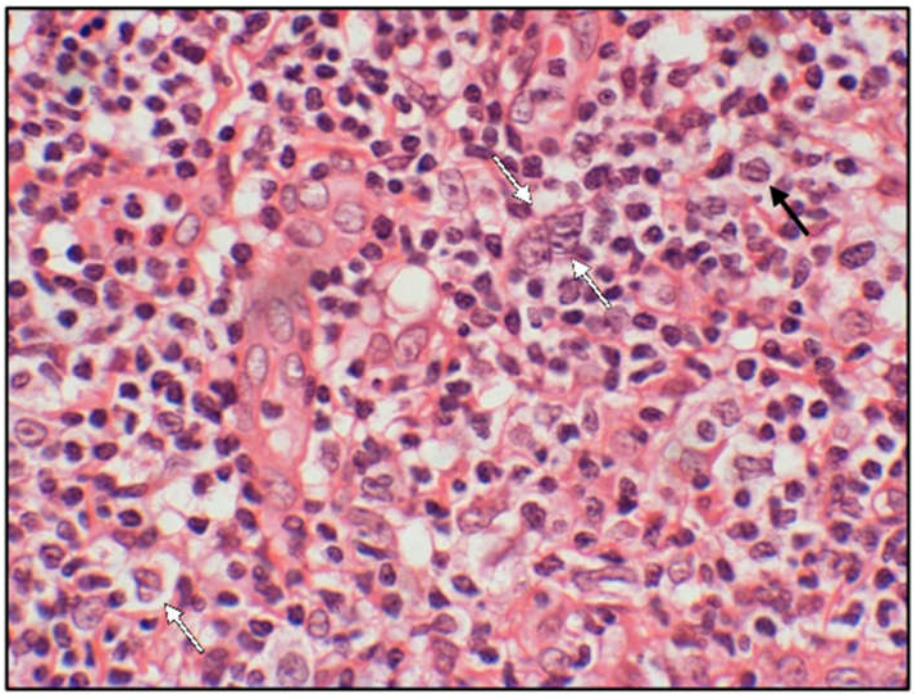

Figure 1. Hematoxylin-eosin stain. Hodgkin's lymphoma with multinucleated Hodgkin's and Sternberg-Reed cells (arrows)

Regarding Non-Hodgkin's lymphomas, currently WHO distinguishes more than 50 different subtypes, which show broad clinical, epidemiological and pathological heterogeneity. For this reason, only a few sub-forms that are important in terms of common entities (10,16,17)

The NHL subtypes are widely varied with regard to cytomorphologically and histopathologically (11) The diffuse large B-cell lymphoma (Figure 2) is the commonest, contributes for about 30-40% of NHL. In low-grade NHL, follicular subtype id the commonest contributes for 20-25% (18). Subtypes like indolent lymphoma , Burkitt lymphoma and others are less common. Immunophenotypically, membrane-bound IgM, B-cell-specific antigens (CD19, CD20, CD79a) as well as CD5, CD23 and CD43 can be found in B-CLL.

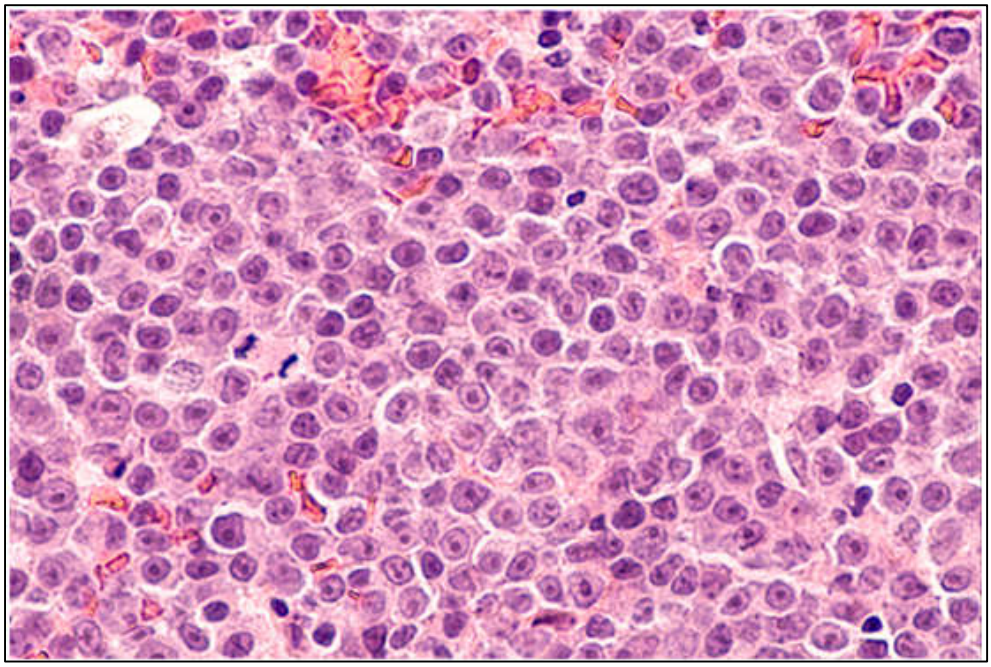

Figure 2. Demonstration of a slide for Diffuse large B-cell Lymphoma

Staging :

The Ann Arbour classification is used to stage Hodgkin's lymphomas and the most of non-Hodgkin's lymphomas. (19). This is based on the anatomical distribution of the affected lymph node regions in the body:

Stage I: only one lymphatic site is affected.

Stage II: In this stage the ≥ 2 LN regions on one side of diaphragm are affected

Stage III: When 2 or more LN regions are affected on both diaphragm's sides

Stage IV: Involvement of multiple regions (diffuse involvement of organs /tissues)

Diagnosis

The first step in diagnosis is the physical examination during which the lymph nodes are palpated. A blood test is also one of the diagnostic measures. In many cases, a tissue sample must be taken to confirm the diagnosis. For this purpose, a suspicious lymph node or suspicious tissue is removed in a small surgical procedure; Depending on the localization, this procedure is performed under local or a short general anesthesia. The sample taken is then examined for histological (histological, immunohistochemical) examination. Some forms of lymphoma are diagnosed from the blood without the need for bone marrow tests (2,20–23).

Immunohistochemistry

The histopathological and immunohistochemical evaluation of lymphomas and its differential diagnosis is a crucial aspect of its diagnosis in an academic setting. The hallmark of immunohistochemical diagnosis in classical Hodgkin's lymphoma is the presence of CD30 and CD15 markers. (22,23)

Additionally, Reed-Sternberg (RS) cells express class II histocompatibility antigens and other accessory molecules, including CD27L, CD30, CD54, CD58, CD80, and CD86, which facilitate attraction and interaction with T lymphocytes. The presence of Epstein-Barr virus (EB) DNA in RS cells has been demonstrated through immunohistochemistry techniques. In NHL, an immunohistochemical examination used to clarify whether a B or T cell lymphoma is present; mature B lymphocytes also carry the protein CD20 on their surface when they develop into cancer cells (B lymphoma cells). Then this protein is found in even higher numbers on the cell surface. In contrast, the surface protein CD3 is typical for T-lymphocytes and the cancer cells that develop from them (T-lymphoma cells). Additionally, other investigations are necessary to be performed these include, for example, inflammatory parameters (24). However, many aspects are still unclear, especially since the entities differ significantly from one to another in numerous respects. Therefore, in order to advance knowledge about diagnostic and prognostic role of immunohistochemistry in malignant lymphomas we present this work.

2. Patients , Material and Methods

A prospective clinicopathological and immunohistochemical study conducted during a period of 20 months, in the years 2021-2023. The study included 42 Iraqi patients with different types of malignant lymphomas (Hodgkin's or Non-Hodgkin's) whose biopsied tissue specimens were referred for histopathological studies. However, some patients had more than one specimen, hence, we were able to retrieve a total of 65 paraffin blocks which were prepared and made available for immunohistochemical staining in the laboratory for cytology and pathology studies (Figure 3). The samples were partly from the same tumor or came from different affected sites of a patient.

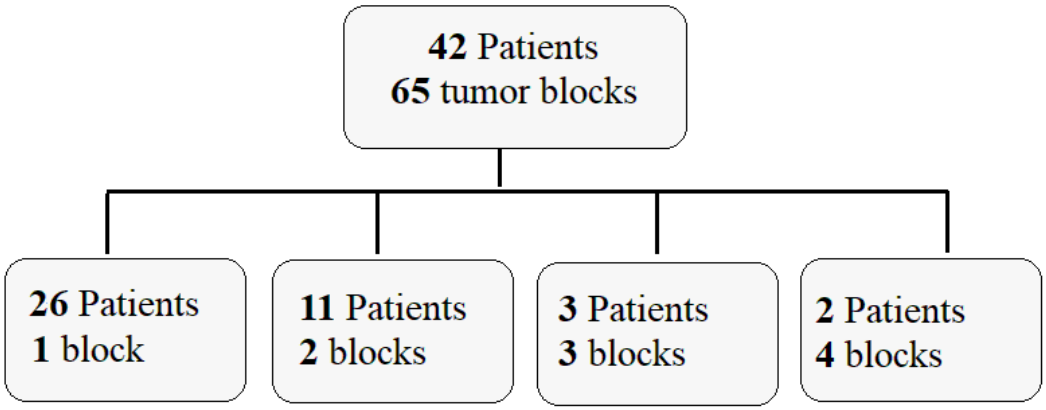

Figure 3. Number of patients and tumor prepared blocks

Inclusion criteria:

1. Iraqi patients with malignant lymphomas
2. Both genders
3. Adult aged older than 18 years
4. Had sufficient one or more specimens of biopsied tissues
5. Tissue samples were exclusively primary tumors
6. Had full-data along with the request for histopathological study

Exclusion criteria:

1. Patients with missed data
2. Patients with incomplete or unapproved diagnosis
3. Patient with recurrence or metastasis

This research project and the associated use of patient data has received an ethical approval from the official authorities and the ethics committee .

Tissue sections and immunohistochemistry

A total of seven tissue sections of 4 µm thick were made from each of the 65 tumor blocks using a microtome and applied to slides coated with poly-L-lysine. and examined using immunohistochemical staining.

Sections from each block were stained with hematoxylin and eosin and analyzed immunohistochemically for different antigens, these include CD3 (B-cell marker), CD20 (as T-

cell marker), CD79 α , CD15, CD30. To support the diagnosis, cHL was considered as CD15+ve, CD30+ve, CD20-ve, while NLPHL type was considered when CD20+ve (25)

After complete drying at room temperature, the immunohistochemical staining was carried out. The deparaffinization process involved immersing the sample in a xylene bath for a duration of 5 minutes, which was repeated for three times. The rehydration process involved immersing the specimen in ethanol baths of varying concentrations (95%, 90%, and 75%) for a duration of 5 minutes each. This was followed by a subsequent immersion in a distilled water bath for an additional 5 minutes. The antigen retrieval process was conducted using a microwave and EDTA buffer (pH= 8) for a duration of 20 minutes. The activity of endogenous peroxidase was suppressed by subjecting the tissue to a 6% oxygenated water solution for a duration of 5 minutes. Subsequently, the specimen is subjected to incubation with primary antibodies for a duration of 60 minutes at a temperature of 37°C, following a prior washing step with a phosphate-buffered saline (PBS) solution for a duration of 5 minutes. The primary antibodies employed in this study were diluted at a ratio of 1:50. Subsequently, the tissues underwent a washing procedure using PBS/Tween solution, followed by incubation at 37°C for 30 minutes using the HPR EnVision detection system. Then the tissues underwent a water wash, which was afterwards followed by signal visualization using 3-3-diaminobenzidine. Subsequently, the nuclei were counterstained using hematoxylin. A dehydration procedure then conducted utilizing a bath containing ethanol of progressively higher concentrations by a cleansing and mounting procedure involving the application of Canadian balsam and coverslips. Antigens in a tissue can be made visible with the help of immunohistochemistry. In the present work, the indirect peroxidase method was used to detect the Antigens.

If a patient had several tumor blocks, an average was calculated from the individual values, so that at the end , exactly one IRS value per receptor and one Ki-67 index was determined for each patient.

Evaluation of the immunohistochemical staining

The determination of the percentage of cells that exhibit positive staining was initially conducted using the light microscope (Olympus, Tokyo, Japan) which provided the basis for the evaluation of the stained preparations. The focus was on color intensity, staining proportion, type of staining, membrane permanence, vascularization, distribution and

expression patterns of stained areas as well as stained areas in the surrounding tissue. Immunoreactive Scores were evaluated using the 12-points semiquantitative immunoreactivity score (IRS) (ranging from 0 to 12 points) was used to figure out how strong the cytoplasmic reaction was. (Table 1).

Table 1. Semi quantitative immunoreactivity score (IRS)

Positivity of cells		**Staining reaction Intensity**	
Score	Percent	Score	Reaction intensity
0	None	0	None
1	= 10 %	1	Weak
2	11 - 50%	2	Moderate
3	51 - 80%	3	Strong
4	> 80 %		

IRS = Positivity of cells % x Staining reaction Intensity

0 - 2 points: Negative

3 - 5 points: weak

6 - 8 points : Moderate

9 - 12 points : Strong

The immunoreactivity score (IRS) is calculated as the product of multiplication of the percentage of positivity of cells by the staining reaction intensity. The values of IRS were categorized as follows: 0-2, 3-5, 6-8 and 9-12to indicate negative, weak, moderate or strong positivity, respectively.

Assessment of Proliferation activity with Ki67

The Ki67 proved to be a valuable predictor of lymphoma with some variation across the subtypes (26). Also it had been documented that Ki67 index of ≤ 45% can differentiate indolent from aggressive lymphomas and the higher Ki67 index associated with more advanced grade . Therefore, the proliferation activity of the malignant lymphomas, was assessed depending the proportion of cell nuclei which are positive for Ki67. The same

immunohistochemical procedure as described before was applied. At 400x magnification, the positive and negative cell nuclei were counted manually from 10 main visual fields each from a representative tumor area. Subsequently, we calculated the Ki-67 proliferation index according to the following equation (26–28):

$$\frac{\text{Number of Ki-67 positive nuclei}}{\text{Total Number of tumor cells}} \times 100\%$$

Statistical analysis:

The statistical tests were performed with SPSS version 28. First, the data were checked for normality using the Kolmogorov-Smirnov test of goodness.

A normal distribution could only be determined for the age of the patients when the disease was first diagnosed. The Mann-Whitney U test was used to find out whether there was a difference between the IRS values of the HL and the NHL. Chi-square test used to compare categorical variables across the subgroups. Exact tests used accordingly when the chi square was inapplicable . Receiver operating characteristics (ROC) curve analysis used to assess the validity of IRS and Ki-67 in prediction of advanced stages and prognostic values. P. value of ≤ 0.05 considered significant.

3. RESULTS

During the study period, 42 patients with malignant lymphomas were diagnosed HL found in 24 (57.1%) and NHL in 18 patients (42.9%), (Figure 4), Among the cases, males were relatively dominant contributed for 54.8% (Figure 5). Age distribution showed a mean of 47.3 ± 9.1 years in HL group and 50.6 ± 8.4 years in NHL group with no significant difference, (P>0.05). Males were dominant in both HL and NHL groups, contributed for 54.2 and 55.6%, respectively, (P > 0.05), (Table 1). Nodular sclerosis was the more frequent HL subtype (45.8%) followed by Mixed cellularity (41.7%), Lymphocyte-dominant and lymphocyte-depleted HL were found in 8.3% and 4.2%, respectively. Among NHL group, B-cell NHL was the commonest (72.2%), T-cell type in 22.2% while mixed type in only one patient (5.6%). Ann Arbor staging revealed stage I in 8 patients, stage II in 16, stage III in 11 and stage IV in 7 patients, contributed for 19%, 38.1%, 26.2% and 16.7%, respectively, (Table 2).

Table 3 summarizes the laboratory parameters that received with the histopathology requests. A total of 65 tissue specimens were received belonged to the 42 cases of lymphoma where some patients had more than one specimen. The tissue specimens were biopsied from lymph nodes of cervical regions in 29.2%, axillary regions (26.2%) and inguinal regions (18.5%). Specimens of bone morrow biopsy represented 15.4% of all specimens. Mesenteric/peritoneal and mediastinum /pulmonary specimens were 4 and 3 respectively, however, some of the different tissue samples were taken from the same lymph node, some of the samples came from different affected lymph nodes or tissues, (Table 4).

The CD and Ki-67 expression in the studied specimens revealed positive CD3 expression in 35.4%, CD15 (93.8%), CD20 (83.1%), CD30 (95.4%), and CD79α in 69.2%. Ki-67 expression of ≥ 45% was reported in 33.8% and < 45% in 66.2%, (Table 5). Immunoreactivity score (IRS) of the 65 tissue specimens revealed low score of 0-2 in 11 specimens (16.9%), a score of 3 – 5 reported in 23 (35.4%), IRS of 6-8 in 19 (29.2%) and a score of 9-12 reported in 12 specimens (18.5%), (Table 6). For assessment of the role of IRS and Ki-67 in prediction of advanced stage lymphomas , we used receiver operating characteristics (ROC) curve analysis , (Figure 6 and 7), which revealed that both IRS and Ki-67 were good and reliable predictors for advanced stage of lymphomas. For IRS the area under the ROC curve (AUC) was 0.908 at a cutoff point of ≥ 5 and produced a sensitivity of 85.3%, specificity of 86.3%, and accuracy of 85.8%. For Ki-67 index the AUC was 0.847 at a cutoff point of > 45% giving a sensitivity, specificity and accuracy of 85.7%, 89.8% and 87.8%, respectively, (Table 7).

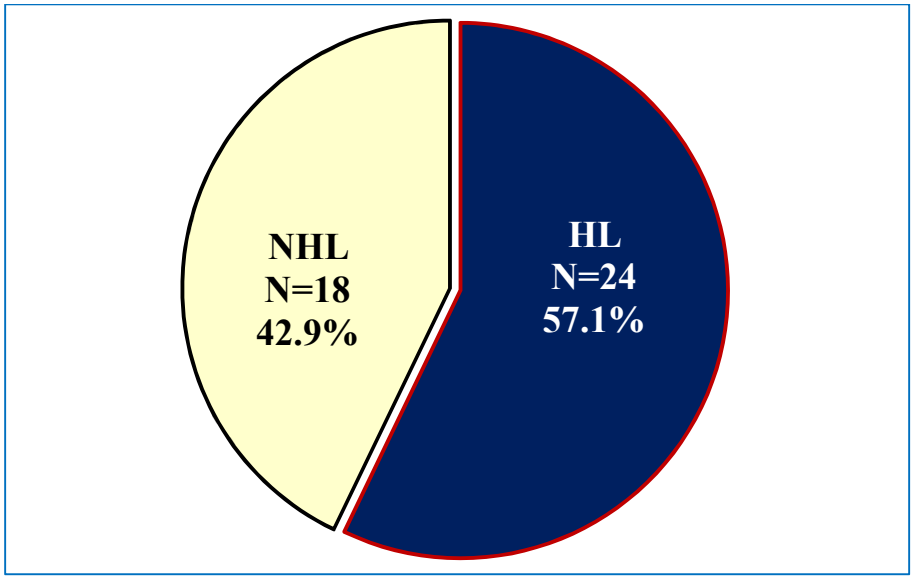

Figure 4. Distribution of the studied group according to the type of lymphoma (N= 42)

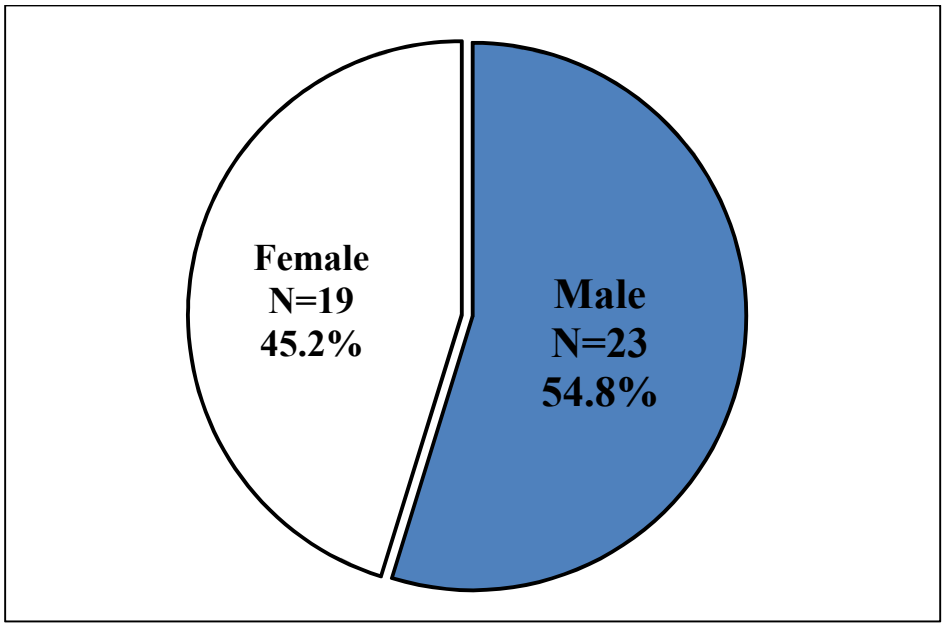

Figure 5. Distribution of the studied group according to gender (N= 42)

Table 1. age and gender distribution according to the type of lymphoma

Variable		HL (N= 24)		NHL (N= 18)		P. value
		No.	%	No.	%	
Age (year)	< 30	13	54.2	6	33.3	0.330 ns
	30 - 39	6	25.0	5	27.8	
	≥ 40	5	20.8	7	38.9	
	Mean age (SD)	47.3 (9.1)		50.6 (8.4)		0.236 ns
Gender	Male	13	54.2	10	55.6	0.892 ns
	Female	11	45.8	8	44.4	

SD: Standard deviation, ns: not significant

Table 2. Distribution of Lymphoma cases according to the subtypes

Lymphoma	Subtype	No.	%
Hodgkin's lymphoma	Nodular sclerosis	11	45.8
	Mixed cellularity	10	41.7
	Lymphocyte-dominant	2	8.3
	Lymphocyte-depleted	1	4.2
Total		24	100.0
Non- Hodgkin's lymphoma	B-cell	13	72.2
	T-Cell	4	22.2
	Mixed (non-specified)	1	5.6
Total		18	100.0
Ann Arbor staging	Stage I	8	19.0
	Stage II	16	38.1
	Stage III	11	26.2
	Stage IV	7	16.7
Total		42	100.0

Table 3. Distribution of laboratory parameters of 42 cases with malignant lymphomas

Parameter		No.	%
HGB	< 11	10	23.8
	≥ 11	32	76.2
WBC (x10³) cells/mm³	< 15	34	81.0
	≥ 15	8	19.0
Lymphocyte (x10³) cells/mm³	< 0.7	3	7.1
	≥ 0.7	39	92.9
ESR	< 30	13	31.0
	≥ 30	29	69.0
LDH	< 450	30	71.4
	≥ 450	12	28.6
Bone morrow	Involved	4	9.5
	Not involved	38	90.5

Table 4. Sites of 65 tissue specimens from 42 cases with malignant lymphomas*

Site	No.	%
Cervical regions	19	29.2
Axillary regions	17	26.2
Inguinal regions	12	18.5
Bone marrow biopsy	10	15.4
Mesenteric/peritoneal	4	6.2
Mediastinum /pulmonary	3	4.6
Total samples	65	100.0
*Some cases had more than one specimen,		

Table 5. CD and Ki67 expression in 65 tissue specimens from 42 cases with malignant lymphomas

Marker	Status	No.	%
CD3	Positive	23	35.4
	Negative	42	64.6
CD15	Positive	61	93.8
	Negative	4	6.2
CD20	Positive	54	83.1
	Negative	11	16.9
CD30	Positive	62	95.4
	Negative	3	4.6
CD79α	Positive	45	69.2
	Negative	20	30.8
Ki67 expression	≥ 45 %	22	33.8
	< 45 %	43	66.2

Table 6. Immunoreactivity score (IRS) of the 65 tissue specimens from 42 cases with malignant lymphomas

IRS	No.	%
0 - 2	11	16.9
3 – 5	23	35.4
6 – 8	19	29.2
9 - 12	12	18.5
Total	65	100.0

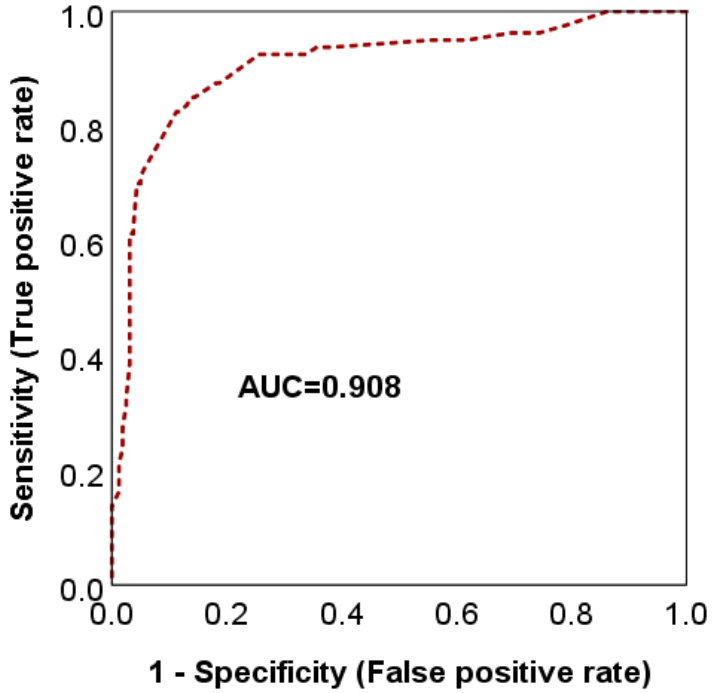

Figure 6. ROC analysis for the validity of IRS in prediction of advanced stage of lymphomas

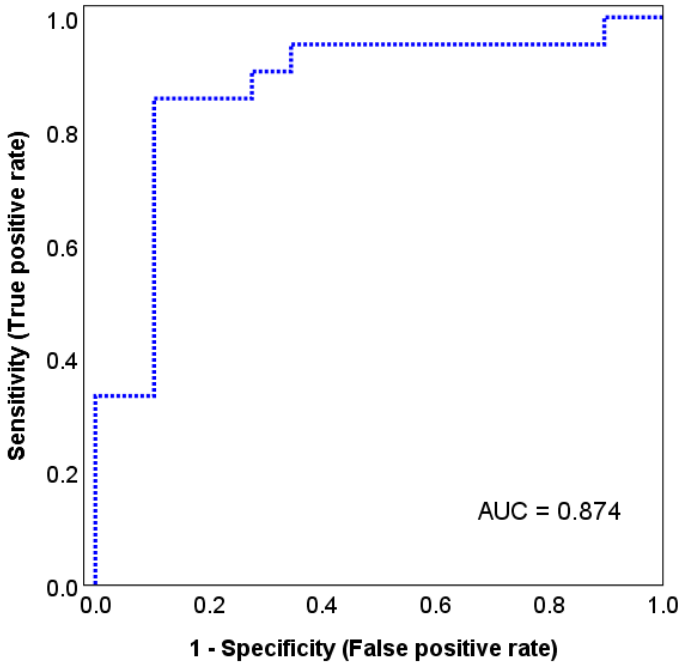

Figure 7. ROC analysis for the validity of Ki-67 in prediction of advanced stage of lymphomas

Table 7. Cutoff points and validity parameters of Ki67 index and IRS in prediction of advanced stage and prognostic cutoff points of malignant lymphomas

Parameter	IRS	Ki67 index
Cutoff point	≥ 5	> 45%
AUC	0.908	0.874
Sensitivity	85.3%	85.7%
Specificity	86.3%	89.8%
Accuracy	85.8%	87.8%
PPV	86.2%	89.4%
NPV	85.4%	86.3%

AUC: Area under the ROC curve, PPV: positive predictive value, NPV: Negative predictive value.

4. DISCUSSION

The nomenclature "malignant lymphoma" was initially introduced by Bilroth in 1871 to delineate the presence of neoplasms originating from lymphoid tissue, nonetheless, it appeared that the lymph node enlargement may arise from a combination of neoplastic, infective, and other miscellaneous factors (29). Since that time, malignant lymphomas represents a heterogeneous collection of neoplasms characterized by tumour cells that have similarity to mature lymphocytes, histiocytes, or their progenitors. Lymphomas are categorized into two distinct types: Hodgkin lymphoma (HL), which exhibits a relatively defined nature, and non-Hodgkin lymphoma (NHL), which displays a significantly greater degree of heterogeneity. The conventional approach to diagnosis, classification, and prognostic evaluation relies solely on morphological characteristics such as cell size, nuclear and cytoplasmic features, and the distribution pattern (nodular or diffuse). However, this method has inherent limitations and necessitates the use of additional techniques such as immunohistochemistry, cytogenetics, and molecular studies to achieve a more precise and accurate diagnosis (30). The current study tried to assess the characteristics of malignant lymphomas among Iraqi patients who had this malignancy and to achieve a comprehensive examination of the immunodiagnostic approach to lymphoma, along with an exploration of

some prevalent immunohistochemistry markers employed in this context. A total of 65 tissue specimens were received in our lab belonged to 42 patients with different types of malignant lymphomas. We only included the specimens that exclusively taken from primary lesions. Despite the fact that the incidence of HL is lower than NHL worldwide and the incidence of HL either decreases or stationary in many countries (4), we found higher rate (57.1%) of HL among our cohort, however, this could be due to difference in the nature of malignant lymphomas in Iraqi population where previous Iraqi studies documented higher prevalence of HL than NHL among Iraqi population , Ghazi et al. found HL in 62% of their studied group (31). We found no significant difference in age or gender distribution across the types of lymphoma, HL vs. NHL, however, our objectives did not concern with the age or gender variation in malignant lymphomas, nonetheless, previous studies have shown predominance of male gender in HL and higher incidence of NHL with advancing age, however, with a few exceptions, malignant lymphomas are to be regarded as diseases of old age, which is also reflected in the patient data examined. (4). This may be attributed to our small sample size. In our study, Nodular sclerosis and Mixed cellularity were the commonest HL subtypes while B-cell subtype was the most common NHL which agreed that reported in previous studies (31,32). We documented that cervical regions and axillary regions were the commonest sites from which the specimens were selected, followed by inguinal regions. It is well known that malignant lymphomas are more common in these regions (33). In our study, the expression of expression of CD30 reported in (95.4%) which is close to the findings of Fadhil et al. (32) who found expression of CD30 in 100% however, we found CD15 expressed in almost 94% of cases which is higher than that reported by Fadhil et al. (32) who found CD15 expressed in almost 51%, We found that CD79α expressed in 69.2% of cells. Lower expression rate reported in HL as reported by Sakatani et al. who found that CD79α expressed in 36.4% of HL(34). However, the expression of CDs is widely varied among different studies and may affected by the variation in the populations, the study designs, the laboratory techniques and the procedures applied.

In the current study we found that Immunoreactivity score (IRS) and Ki-67 proliferative index were good and reliable predictors for advanced stage of lymphomas. For IRS the area under the ROC curve (AUC) was 0.908 at a cutoff point of ≥ 5 and produced a sensitivity of 85.3%,

specificity of 86.3%, and accuracy of 85.8%. For Ki-67 index the AUC was 0.847 at a cutoff point of > 45% giving a sensitivity, specificity and accuracy of 85.7%, 89.8% and 87.8%, respectively. These findings reflect the value and role of immunohistochemistry in diagnosis and prognosis of malignant lymphoma in both of their types , HL and NHL. Similar findings were also reported in earlier studies; Lacet et al. documented that lower positivity CD cells associated with better prognosis and longer disease free rate and survival (22). In a study conducted in China, HE et al. documented that Ki-67 proliferation index is a valuable predictor of lymphoma with some variation across the subtypes (26). On the other hand, the Chinese study proved that Ki67 index of ≤ 45% can differentiate indolent from aggressive lymphomas and the higher Ki67 index associated with more advanced grade as we did find in our study. Zeggai et al. concluded that immunohistochemistry study can be useful in monitoring and follow-up of patients at risk and help to achieve appropriate management (35). Cho et al. concluded that IHC studies are important in diagnosis, staging and prognosis of malignant lymphomas.

Limitations of our stud are due to small sample size which is attributed to the low incidence of malignant lymphoma in general and lower number of patients who were registered in our center. However, further studies included multiple center are highly recommended to include larger number of patients with this disease.

5. CONCLUSIONS

The immunohistochemical (IHC) studies has a crucial role in the diagnostic process of types and subtypes of lymphomas. Immunoreactivity score (IRS) and Ki-67 proliferative index were good predictor of advanced stage malignant lymphomas for both HL and NHL. The selection of suitable markers by pathologists is of utmost importance, as it should align with the patient's clinical condition, and the outcomes of immunohistochemistry studies, and the precise interpretation of immunohistochemistry data to achieve an optimal diagnosis of lymphoma. Hence, it is imperative for physicians to ensure the collection of adequate samples and appropriate fixation techniques in order to achieve precise immunohistochemistry results that can contribute to a conclusive and dependable pathology diagnosis.

Ethical Approval:

All ethical issues were approved by the author. Data collection and patients enrollment were in accordance with Declaration of Helsinki of World Medical Association , 2013 for the ethical principles of researches involving human. Signed informed consent was obtained from each participant and data were kept confidentially.

6. BIBLIOGRAPHY

1.Lymphoma Action Team. Types of Lymphoma. Availablehttps//lymphoma-action.org.uk/types-lymphoma Accessed July 2023.

2.Lewis WD, Lilly S, Jones KL. Lymphoma: diagnosis and treatment. Am Fam Physician. 2020;101(1):34–41.

3.de Leval L, Jaffe ES. Lymphoma classification. Cancer J. 2020;26(3):176–85.

4.Huh J. Epidemiologic overview of malignant lymphoma. Korean J Hematol. 2012 Jun;47(2):92–104.

5.Johnson PWM. Are we reaching the maximum cure rate for Hodgkin lymphoma? Hematol Oncol. 2023;41:57–61.

6.Armitage JO, Longo DL. Mantle-cell lymphoma. N Engl J Med. 2022;386(26):2495–506.

7.Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment ofHodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin OncolOff J Am SocClin Oncol. 2014 Sep;32(27):3059–68.

8.Dürkop H, Foss H-D, Stein H. 2008-WHO-Klassifikation der malignen Lymphome im Jahr 2011. Der Onkol. 2011;17(9):769–88.

9.Ansell SM. Hodgkin lymphoma: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol. 2022;97(11):1478–88.

10.Jaffe ES. World Health Organization classification of tumours. Pathology & genetics. Tumours of hematopoietic and lymphoid Tissues. IARC Press Lyon. 2001;

11.Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, J Am Soc Hematol. 2016;127(20):2375–90.

12.Pileri SA, Ascani S, Leoncini L, Sabattini E, Zinzani PL, Piccaluga PP, et al. Hodgkin's lymphoma: the pathologist's viewpoint. J Clin Pathol. 2002 Mar;55(3):162–76.

13.Küppers R, Engert A, Hansmann M-L. Hodgkin lymphoma. The Journal of Clinical Investigation, 2012; 122 (10):3439-3447.

14.Takahara T, Satou A, Tsuzuki T, Nakamura S. Hodgkin Lymphoma: Biology and Differential Diagnostic Problem. Diagnostics. 2022;12(6):1507.

15.Piccaluga PP, Agostinelli C, Gazzola A, Tripodo C, Bacci F, Sabattini E, et al. Pathobiology of Hodgkin Lymphoma. Tobinai K, editor. Adv Hematol. 2011;2011:920898.

16.Koff JL, Chihara D, Phan A, Nastoupil LJ, Williams JN, Flowers CR. To each its own: linking the biology and epidemiology of NHL subtypes. Curr Hematol Malig Rep. 2015;10:244–55.

17.Chihara D, Nastoupil LJ, Williams JN, Lee P, Koff JL, Flowers CR. New insights into the epidemiology of non-Hodgkin lymphoma and implications fortherapy. Expert Rev Anticancer Ther. 2015 May;15(5):531–44.

18.Carbone A, Roulland S, Gloghini A, Younes A, von Keudell G, López-Guillermo A, et al. Follicular lymphoma. Nat Rev Dis Prim. 2019;5(1):83.

19.Zschoche M, Zimpfer A, Scheef BO, Jünemann AM, Guthoff RF, Junghanss C, et al. Histopathological features and Ann Arbor stage in periocular lymphoma. In Vivo (Brooklyn). 2020;34(4):1965–74.

20.Storck K, Brandstetter M, Keller U, Knopf A. Clinical presentation and characteristics of lymphoma in the head and neck region. Head Face Med. 2019;15(1):1–8.

21.Mezger NCS, Feuchtner J, Griesel M, Hämmerl L, Seraphin TP, Zietsman A, et al. Clinical presentation and diagnosis of adult patients with non-Hodgkin lymphoma in Sub-Saharan Africa. Br J Haematol. 2020;190(2):209–21.

22.Lacet DFR, Oliveira CC. The role of immunohistochemistry in the assessment of classical Hodgkin lymphoma microenvironment. Int J Clin Exp Pathol. 2022;15(10):412.

23.Goyal A, Casillo C, Narayanan D, Pinkus GS, Russell-Goldman E. Initial Diagnosis of Classic Hodgkin Lymphoma With Skin Biopsy: A Rare Case and Review of Diagnostic Considerations. Am J Dermatopathol. 2023;45(8):577–81.

24.Cho J. Basic immunohistochemistry for lymphoma diagnosis. Blood Res. 2022 Apr;57(S1):55–61.

25.von Wasielewski R, Mengel M, Fischer R, Hansmann ML, Hübner K, Franklin J, et al. Classical Hodgkin's disease. Clinical impact of the immunophenotype. Am J Pathol. 1997 Oct;151(4):1123–30.

26.He X, Chen Z, Fu T, Jin X, Yu T, Liang Y, et al. Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies inlymphoma subtypes: evidence from a systematic meta-analysis. BMC Cancer. 2014 Mar;14:153.

27.Maeshima AM, Taniguchi H, Hori Y, Ida H, Hosoba R, Makita S, et al. Diagnostic utility and prognostic significance of the Ki-67 labeling index in diffuse large B-cell lymphoma transformed from follicular lymphoma: a study of 76 patients. Pathol Int. 2021;71(10):674–81.

28.Meyer H-J, Wienke A, Surov A. Correlations between imaging biomarkers and proliferation index Ki-67 in lymphomas: a systematic review and meta-analysis. Clin Lymphoma Myeloma Leuk. 2019;19(6):e266–72.

29.Lakhtakia R, Burney I. A Historical Tale of Two Lymphomas: Part II: Non-Hodgkin lymphoma. Sultan Qaboos Univ Med J. 2015 Aug;15(3):e317-21.

30.Nirmal RM. Diagnosis of malignant lymphoma - An overview. J Oral Maxillofac Pathol. 2020;24(2):195–9.

31.Ghazi HAA-M, Al-Taee RAM, Al-Hindy HA-AM. Immunophenotypic characterization of malignant lymphoma in Iraqi patients using immunohistochemical CD-marker study. Syst Rev Pharm. 2020;11(11):412–7.

32.Fadhil MS, Al-Nueimy WM, Lazim AF. Hodgkin's lymphoma. An immunohistochemical profile in northern Iraq. Saudi Med J. 2014 May;35(5):448–53.

33.Kami TS, Al-rawi RAR, Alhaideri M. Immunohistochemical and clinicopathological study of non-Hodgkin's lymphoma in Erbil, Kurdistan. Zanco J Med Sci (Zanco J Med Sci). 2014;18(2):756–62.

34.Sakatani A, Igawa T, Okatani T, Fujihara M, Asaoku H, Sato Y, et al. Clinicopathological significance of CD79a expression in classic Hodgkin lymphoma. J Clin Exp Hematop. 2020 Sep;60(3):78–86.

35.Zeggai S, Harir N, Tou A, Sellam F, Mrabent MN, Salah R. Immunohistochemistry and scoring of Ki-67 proliferative index and p53 expression in gastric B cell lymphoma from Northern African population: a pilot study. J Gastrointest Oncol. 2016;7(3):462.

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